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CHAPTER 30
Animal Models of Dyspareunia

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Introduction

In recent years, the development and application of new animal models of disease processes has been a popular scientific trend [1]. However, few of these models have focused on sexuality, and fewer still have modeled pain conditions that impact sexuality. Urogenital and abdominal pain conditions associated with dyspareunia impact a staggering percentage of women, yet very few of these conditions are well understood. Although imaging studies have greatly advanced human research in this area [2], experimental options using human subjects are still limited. Animal models allow experimental manipulations to evaluate the causal relationships between pathological causes and physiological effects. These models are convenient and cost-effective, and they permit the testing of hypotheses that are otherwise ethically implausible in humans. The development of viable animal models for conditions that are associated with painful intercourse, such as endometriosis, interstitial cystitis (IC), irritable bowel syndrome (IBS), and provoked vestibulodynia (PVD) might have profound implications for our understanding of the etiology, maintenance, and treatment of these debilitating conditions.

Evaluation of Animal Models of Pain

Pain in animals is defined as "an aversive sensory experience caused by actual or potential injury that elicits progressive motor and vegetative reactions, results in learned avoidance behavior, and may modify species-specific behavior, including social behavior" [3]. Animals cannot verbally rate their pain intensity, quality, or location, nor can they communicate the impact of emotion on pain. Instead, researchers infer the presence of pain from abnormal behaviors that are (hopefully) unique to the experimentally induced nociceptive state. The difficulty in measuring an animal's emotional or cognitive responses to pain suggests that we are largely using *nociceptive models*, rather than true pain models [4]. However, just because we can't measure something doesn't mean it isn't there. Nevertheless, the word *pain* will be used throughout this chapter.

Pain can be typified as spontaneous or provoked, depending on whether or not it is elicited by exogenous stimulation. Many existing animal models of pain are limited in their duration; chronic, spontaneous pain—the most clinically relevant form—has proved particularly difficult to model in animals [5]. Pain conditions can also be visceral or somatic in nature. Visceral pain originates from the internal organs contained within the chest and abdomen, and it is characterized by increased autonomic reactivity, emotional salience, and diffuse pain that may be referred to other visceral or somatic tissue that shares common innervation at the level of the spinal cord [6]. Referred pain is perceived in areas distal from the site of injury that receives common spinal input as the region where pain originates [7, 8]. The majority of animal models of pain conditions associated with dyspareunia are visceral in nature, including uterine inflammation, vaginal and uterine distension, endometriosis, and abdominal pain (including cystitis and colitis).

In animal models, behavioral responses may reflect the location of the pain in the case of somatic tissue (e.g., withdrawal of a heated hind paw), whereas visceral pain

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may be manifested as referred somatic pain [7]. Patterns of pain behavior can increase in frequency or magnitude with higher levels of noxious stimulation. Abnormal behaviors that show temporal correspondence with tissue injury or inflammation are thought to reflect injury-specific pain, although the correlation may not be strong. For visceral pain in particular, the *absence* of behavior may be indicative of pain, as evidenced by reduced mobility or motivation to engage in normal activity [9]. In addition, estrous cyclicity may significantly impact some behaviors [10], but many indices of behavioral pain show equal variability when male versus female animals are used [11]. Empirical validation that a behavior is specific to pain is often achieved via the administration of known analgesics, such as nonsteroidal anti-inflammatory drugs, lidocaine, or morphine.

Ultimately, many behaviors have been associated with pain in rodents. Table 30.1 lists behaviors that have been linked to animal models of dyspareunia. Notably, the criteria for dyspareunia vary between models. Whereas some models directly measure vaginal sensitivity to noxious stimuli, other models induce pain conditions associated with dyspareunia. Ideally, pain behaviors are unique to an experimental manipulation, easily quantifiable with minimal need for interpretation, frequent enough to allow for statistical comparisons between groups, and reliably observed in afflicted animals (and rare in healthy animals). Such behavior should coincide with the duration and severity of injury and be mitigated by analgesics in a dose-dependent manner. Most importantly, the validity of an animal model of pain relies on whether the researchers have accurately identified a pain behavior that closely parallels the clinical characteristics of the condition it is intended to model. This chapter will be limited to reviewing rodent models of female urogenital and abdominal pain that include the measurement of pain *behavior*, not electrophysiological or electromyographic proxies, as a primary outcome measure [4].

Animal Models of Dyspareunia

Ureteral Calculosis

Women with dysmenorrhea, or painful menstruation, often report dyspareunia and are more likely to experience urinary calculosis (kidney stones). Based on this comorbidity, animal models of ureteral calculosis (UC) may indirectly induce dyspareunia, although this link has never been formally tested.

The first detailed behavioral characterization of UC-induced visceral pain was conducted by Giamberardino's laboratory [12]. Within a day of implantation of an artificial stone into the left ureter, rats displayed a variety of spontaneous pain behaviors including stretching, hunched back, abdominal/flank licking, flank muscle contractions accompanied by ipsilateral inward hindlimb motions, lower abdominal squashing (against the floor), and the adoption of a supine position with the left hindlimb retracted into the abdomen. These behaviors slowly decreased in frequency and duration over four days postimplantation. Rats with frequent visceral pain behaviors were more likely to vocalize to electrical stimulation of the ipsilateral oblique muscles, indicative of referred pain. These behaviors are similar to the protracted abdominal stretching observed in early visceral pain models [13, 14]. Pain behaviors were reduced with intraperitoneal 5 mg/kg/day morphine. Giamberardino's model established a typology for abnormal pain behaviors associated with visceral pain that would be replicated or modified by the majority of subsequent abdominal visceral pain models.

Based on preliminary human evidence linking dysmenorrhea, endometriosis, and UC [15], Giamberardino and colleagues [16] developed a dual rat model of endometriosis with UC to investigate whether abdominal pain behaviors found in either condition are enhanced by the comorbidity. Animals with endometrial autografts plus stone implantations showed significantly longer bouts of pain behavior compared to stone implantation only or sham groups. Although all animals developed some referred hyperalgesia caused by the presence of a ureteral stone, the endometriosis + UC group displayed the greatest magnitude of referred pain as indicated by reduced vocalization threshold in response to electrical stimulation of the left oblique muscles.

Uterine Inflammation

Wesselmann and colleagues [17] characterized pain behavior associated with uterine inflammation in the rat. The pain behaviors they examined were based on the Giamberardino model of UC [12]. To induce inflammation, 10% mustard oil and a mineral oil vehicle were injected into the uterine lumen, and pain behaviors were videotaped for seven days postsurgery. Of animals receiving

Table 30.1 Common pain behaviors used in animal models of dyspareunia.

Pain Behaviors	Type of Pain	Condition Modeled	References
Pushing abdomen against floor ("stretch-flat" position)	Spontaneous, visceral	Ureteral calculosis, endometriosis, uterine inflammation, colitis, parturition	12, 16, 17, 41, 49
Lifting abdomen off floor	Spontaneous, visceral	Colitis	42–43
Sharp back hunch ("lambda" position)	Spontaneous, visceral	Ureteral calculosis, endometriosis, uterine inflammation	12, 16–17
Abdomen pressed against floor with nose facing toward tail of afflicted side ("alpha" position)	Spontaneous, visceral	Ureteral calculosis, endometriosis, uterine inflammation	12, 16–17
Lower abdomen pressed against floor while standing/sitting ("squash-pelvic" position)	Spontaneous, visceral	Ureteral calculosis, endometriosis, uterine inflammation, parturition	12, 16–17, 49
Stretching (back arched)	Spontaneous, visceral, mechanical distension	Ureteral calculosis, uterine inflammation, uterine distension, cystitis, colitis	12, 17–18, 32, 41-43
Experimenter observed abdominal contractions	Spontaneous, visceral	Cystitis, colitis	32–33, 41–44
Hunched posture	Spontaneous, visceral, mechanical distension	Ureteral calculosis, uterine inflammation, uterine distension, cystitis, parturition	12, 17–18, 31, 33, 35–36, 49
Inward turning of hindlimb	Spontaneous, visceral	Ureteral calculosis, uterine inflammation, parturition	12, 17, 49
Jumping or retreating from palpation/pressure	Provoked, mechanical or thermal or electrical, referred	Ureteral calculosis, referred hypersensitivity from: uterine inflammation, cystitis, colitis, ovariectomy, YIST model	12, 17, 34–35, 37, 41, 43–44, 46–47
Operant response	Provoked mechanical distension, spontaneous, referred	Vaginal and uterine distension, endometriosis, ovariectomy	18, 20–21, 24–25, 29
Licking afflicted area	Spontaneous or provoked	Ureteral calculosis, uterine inflammation, cystitis, colitis, parturition	12, 17, 31–34, 41, 49
Writhing	Spontaneous, visceral, tonic		13–14
Reduced physical activity	Spontaneous	Cystitis, uterine inflammation, colitis	7, 17, 32, 41–42
Vocalization	Spontaneous or provoked	Ureteral calculosis + endometriosis, uterine inflammation, uterine distension	12, 16–18
Piloerection	Spontaneous	Cystitis (rat model only)	31–35
Abnormal defecation/urination	Spontaneous or provoked	Colitis	43
Facial expression (eye squint, blink)	Spontaneous	Cystitis	36

uterine inflammation, 79% displayed prolonged periods of spontaneous pain behavior, with behavior frequency peaking two days after surgery. Dramatic individual differences were found in the frequency and duration of pain behaviors, and animals with uterine inflammation showed reductions in overall mobility. Of animals displaying spontaneous pain behavior, 66% also showed referred muscle hypersensitivity in the lower back and flanks that actually outlasted the occurrence of spontaneous pain behaviors.

Wesselmann's study was especially significant in that it established that pain from distinct viscera—the ureter and the uterus—resulted in very similar behaviors, including behavioral evidence of referred pain. Although this behavioral similarity may support the validity of these behaviors as being specific to pain, it also indicates that the behaviors are not specific enough to distinguish between visceral pains of different origins. The poor localization of visceral pain, however, makes it very unlikely that different visceral pains would be manifested in unique behavioral patterns.

Vaginal and Uterine Distension

Berkley and colleagues [18] established one of the earliest rat models of reproductive tract pain using vaginal and uterine distension. The elegance of this model relies on the novel operant task devised by the authors, which required rats to learn that a discrete behavioral response (extending the nose to interrupt a photocell circuit) would terminate an aversive stimulus (vaginal or uterine mechanical distension with a latex balloon). The authors argued that the rats' motivation to perform the escape behavior in response to high levels of distension indicated that intense mechanical distension constituted an aversive stimulus to the rats. The intense level of stimulation employed by this model is in contrast to innocuous levels of vaginal stimulation, which have positively reinforcing and analgesic properties in rodents [19].

Berkley et al. [18] validated this behavioral pain model in adult virgin female rats with low levels of ovarian hormones (i.e., Metestrus), to control for the potentially confounding effects of estrous cycle hormone fluctuations. Rats reliably escaped distension with increasing speed and frequency as the vaginal distension volume increased, and this response pattern held throughout the estrous cycle [20]. The rats' ability to detect and escape from uterine distension, however, was less predictable—many rats produced operant responses during control trials when distension volumes were minimal, and a large minority of animals did not show behavioral discomfort with maximum levels of uterine distension. The authors noted that rats often responded to uterine distension with stretching behavior.

Interestingly, escape behaviors increased in response to higher vaginal and uterine pressures when estrogen levels were low during Metestrus and Diestrus [20]. Similarly, ovariectomy (OVX) also induced moderate to high levels of vaginal hyperalgesia that were promptly reversed with 17β-estradiol replacement [21]. This pattern of estrogen-dependent vaginal sensitivity has adaptive reproductive significance. The increased tolerance to vaginal pressure, such as that induced by penile penetration, would be functionally important during the height of sexual activity in late proestrus, after estrogen and progesterone levels have peaked.

The development of this model exemplifies the successes and hazards of validating reliable behavioral correlates of pain. The authors succeeded in identifying a reliable pattern of behaviors for vaginal distension, yet uterine distension pain proved more difficult to characterize. Escape responding during uterine distension correlated with a prominent visceral pain behavior, which lends support to the aversive quality of the distension stimulus. One strength of this model is that it relies on an organized motor response that requires cerebral processing, which is thought to more accurately reflect the sensory perception of pain compared to simple reflex responses [4].

Endometriosis

Endometriosis is a painful condition defined by dysmenorrhea, dyspareunia, infertility, and chronic abdominal and low back pain [22]. To induce endometriosis, a segment of uterine horn is removed (i.e., hysterectomy), and pieces of endometrial tissue from the uterine horn (or fat for sham-operated controls) are autotransplanted onto blood vessels in the left ovary, the internal lower abdominal wall, or the cascade mesenteric arteries. Cysts rapidly develop at uterine transplant sites. The endometriosis rat model shares important similarities with endometriosis in women, including pelvic pain, infertility, in vitro and in vivo tissue and cell properties, and treatment responses [23].

Berkley and colleagues [24] combined the distension-induced pain model with the endometriosis rat model.

Animals subjected to the endometriosis surgery showed a significant increase in escape behavior in response to vaginal distension compared to baseline, whereas sham-operated animals without cysts showed no change in behavior. The findings of increased hypersensitivity to vaginal distension in rats with endometriosis have immense clinical relevance given the comorbidity of endometriosis and dyspareunia [22].

In a follow-up study, Cason and colleagues [25] found time- and estrous cycle–dependent changes in distension-induced vaginal hypersensitivity following endometriosis surgery. When postsurgical data from all stages of the estrous cycle were pooled together, the rate of escape responding to vaginal distension steadily increased for eight weeks in proportion to the growth of endometrial cysts. When specific stages of the estrous cycle were examined, rats with endometriosis increased escape responding from vaginal distension during Metestrus, Diestrus, and Proestrus (but not Estrus).

This finding is interesting for two reasons: First, the robust impact of endometriosis on vaginal sensitivity is fully reversed for about a day during the estrous cycle; second, this effect appears to be independent of normal patterns of vaginal hypersensitivity wherein moderate and high levels of estrogen during estrus and proestrus enhance tolerance to vaginal pressure [20]. The difference may be that nonpathological fluctuations in vaginal sensitivity are due to the direct effects of estrogen on vaginal tissue [26–27], whereas the pathological mechanisms underlying endometriosis-induced vaginal hyperalgesia may become centrally mediated [28]. Even a profound drop in ovarian hormones due to OVX does not change endometriosis-induced vaginal hyperalgesia [29], suggesting that either a reduction in estrogen levels does not alter the mechanisms underlying the hyperalgesia or that the capacity for both endometriosis plus OVX to produce hyperalgesia is not additive. Estrogen replacement following endometriosis plus OVX reverses the vaginal hypersensitivity, and this reversal may in part be due to central effects of estrogen [29].

Interstitial Cystitis

Interstitial cystitis (IC) is highly comorbid with dyspareunia and may be accompanied by a burning or aching vaginal pain [30]. Animal models of cystitis use a variety of irritants to induce bladder inflammation, including cyclophosphamide (an antitumor agent), turpentine, and even bacteria.

The cystitis-induced visceral pain model was first developed in the rat [31] and then in the mouse [9, 32]. Following cystitis induction, spontaneous pain behaviors progressively increased in frequency and were correlated with increasing severity of bladder inflammation [31–33]. Cystitis pain behaviors may be more pronounced in the rat compared to the mouse, with the former exhibiting spontaneous abnormal behaviors (i.e., hunched posture, abdominal licking and contractions, reduced locomotion) and the latter exhibiting a general reduction in physical activity [9, 31, 32], although one study found comparable hunching behaviors in the mouse [34]. Rat and mouse models show that cystitis produces referred pain to other areas receiving common innervation, such as the tail, hindpaw, and abdomen [9, 34–36]. In both species, cystitis-induced referred mechanical and thermal hypersensitivity were dose-dependently reduced with morphine [9, 34, 35]. The development of cystitis-induced behaviors does not vary across estrous stages, but interestingly, the onset of pain behaviors progresses more rapidly in female compared to male rats [33].

A model of bacteria-induced cystitis demonstrated that mice showed reduced hindpaw-withdrawal latencies to noxious radiant heat for 14 days following *Escherichia coli* administration, whereas otherwise genetically similar mice but with deficient Toll-like receptor 4 (TLR-4) function failed to show this thermal hypersensitivity [37]. Toll-like receptors are part of the innate immune defence against foreign pathogens, and TLR-4 recognizes bacterial wall components, contributing to nuclear factor-kappa B (NF-κB) activation and subsequent increases in proinflammatory cytokine expression [38]. Central TLR-4 has also been implicated in behavioral hypersensitivity to neuropathic pain [39, 40]. These findings indicate that a bacterium is a sufficient inflammatory irritant to induce experimental, TLR-4-dependent cystitis.

Colitis

In order to model functional abdominal pain like IBS, an animal model of visceral pain from colitis was developed by the Cervero laboratory which measured behavioral responses to colonic irritation from capsaicin and mustard oil [41]. Colonic irritation rapidly and dose-dependently produced abdominal pain behaviors

(i.e., abdominal licking and hunching postures), as well as increased mechanical sensitivity on abdominal, tail, and hindpaw tissues indicative of referred pain. Abdominal pain behaviors were dose-dependently reduced by morphine. Similar models that correlated colonic irritation with increased acute and chronic abdominal pain behaviors showed no apparent structural damage to colonic mucosa [42, 43]. Furthermore, a minority of animals (about one-quarter) may develop chronic mechanical and thermal hypersensitivity lasting up to 16 weeks after severe colitis, indicating the presence of referred pain long after colitis-associated inflammation has resolved [44]. Due to the production of abdominal pain without detectable colonic pathology, these animal models are thought to mimic the clinically important characteristics of IBS, including visceral hypersensitivity and referred somatic pain [45].

Estrogen levels may play an important role in visceral pain. Sanoja and Cervero [46, 47] demonstrated that OVX mice developed robust mechanical, thermal, and visceral allodynia and hyperalgesia in abdominal, hindpaw, and proximal tail skin within a month of OVX surgery. Compared to control groups, the OVX group showed significantly greater numbers of referred visceral pain behaviors following intracolonic capsaicin (including abdominal licking, stretching, squashing, and retractions). This shift in pain sensitivity was reversed with 17β-estradiol replacement. A potential mechanism for this model involves serotonin, which is implicated in the descending inhibitory modulation of pain [48].

Parturition

One of the most commonly encountered forms of visceral pain occurs during labor, when the lower uterus and cervix are stretched and sometimes even torn to permit passage of the offspring. A rat model of parturition pain found that pain behaviors observed in the 1.5 hr preceding birth are similar to behaviors outlined in other animal models of visceral pain [49]. Rats in labor displayed frequent abdominal straining and squashing and an inward turning of the hindpaw, and the rate of these behaviors increased proportionately with labor duration. Systemic oxytocin (10 μg/kg) reduced the labor duration and increased the rate of pain behaviors, which were reduced with epidural morphine (30 ug/10 μL).

Yeast-Induced Sensitization to Touch (YIST) Model

Provoked vulvar pain—involving somatic tissue—is the most common cause of dyspareunia, and yet the majority of existing animal models of pain conditions that cause dyspareunia in women are visceral. We have developed a method of testing vulvar mechanical sensitivity in order to evaluate a mouse model of PVD. The testing method is an adaptation of the classic von Frey [50] psychophysical test and involves stimulation of mouse posterior vulvar tissue, located ventrally from the anogenital ridge, with calibrated nylon monofilaments (0.009–2.0 g). Mice display varying intensities of behavior in response to vulvar stimulation, including sniffing or licking of the vulva, body repositioning, or jumps. Because a rapid, full jump (all four paws off the ground) was the behavior most reliably elicited (albeit at high levels of applied force), we adopted this behavior as the criterion for an aversive response to mechanical stimulation.

Based on multiple reports that women with PVD are significantly more likely to have experienced recurrent vulvovaginal candidiasis (RVVC) [51–53], we developed a mouse model of provoked vulvar pain following three successive vulvovaginal infections with *Candida albicans.* For each infection, mice were vaginally inoculated with yeast, and four days following inoculation the infections were verified and eliminated with seven days of oral fluconazole. Following three weeks of consistently negative cultures from vaginal lavage fluid, mechanical sensitivity measurements were taken and compared to baseline measurements. After three yeast infections, significant differences in vulvar mechanical sensitivity were found between RVVC mice exposed to vulvovaginal yeast compared to fluconazole and saline controls. No changes in hindpaw mechanical sensitivity were found, indicating that increases in sensitivity were specific to the vulvar tissue exposed to yeast. We are hopeful that our model may allow an improved understanding of the mechanisms underlying provoked vulvar pain, as well as the development of novel treatments for clinical use.

Validity of Animal Models of Dyspareunia

As outlined in Table 30.2, the animal models we have reviewed do not meet the proposed criteria for

Table 30.2 Evaluation of the validity of behavioral outcome measures presented by model.

Pain Conditions	Behavior Specific to Condition?	Reliable	Frequently Observed?	Behavior Time Course	Related to Pathology?	Reversible with Analgesics?	Human Condition Modeled?
Ureteral calculosis	No	Yes	Varies between individuals	Onset in 1st day, reduces within 4 days	Unknown	Yes	Urinary calculosis (kidney stones)
Uterine inflammation	No	Yes	Varies between individuals	Onset in 2–4 days of mustard oil	No	N/A	Various uterine pathologies
Vaginal distension	Yes	Yes	Yes	Within sec of noxious distension	Yes, in case of endometriosis	N/A	Vaginal dyspareunia
Uterine distension	Sometimes	No	Varies	Within sec of noxious distension	Unknown	N/A	Unknown
Endometriosis	No	Yes	Yes	Abnormal sensitivity by 1–2 mo	Yes, behavior correlates with cyst growth	Yes	Endometriosis
Cystitis	No	Yes	Yes	Gradually increases in 1–4 hrs	Yes, behavior correlates with bladder inflammation	Yes	Cyclophosphamide-induced cystitis
Colitis	No	Yes	Yes	Onset within 1 hr, several days referred pain	No	Yes	Irritable bowel syndrome
Parturition	No	Yes	Yes	Onset 1.5 hr before birth	N/A	Yes	Labor pain
YIST model	Yes	Yes	Yes	Following three infections	Unknown	N/A	Yeast infection–induced provoked vulvar pain

accurately modeling clinical symptoms of dyspareunia and its associated disorders, as the visceral pain behaviors used in most models are not specific to any particular pain stimulus. Only vaginal distension and vulvar mechanical sensitivity behaviors are unique to a stimulus. Most models produce reliable and frequent pain behaviors, although much individual variation may exist [12, 17, 18].

Pain models vary from acute onset [18] to tonic inflammatory [17, 32] and chronic referred pain [44]. A correlation between behavior and physical pathology is largely absent, with the exception of endometriosis and cystitis models [24, 25, 31–33], and most models are reversible with known analgesics. Each of these models requires substantial development, including a refinement of pain behavior patterns, improved understanding of corresponding physiological pathology, and identification of clinically relevant symptoms specific to dyspareunia.

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